

REMARKS

Status of the claims

Claims 57, 63, 64, 66, 68-71 and 87-90, as shown in the response filed on December 22, 2005, were previously pending and were under consideration.

By virtue of the present response, claim 68 has been amended to depend from pending claim 66.

Also, new claims 91-102 have been added. These claims correspond to previously canceled method claims as follows:

New claim	Previous claim
91	1
92	2
93	3
94	9
95	10
96	11
97	12
98	13
99	14
100	15
101	16
102	17

Thus, no new matter has been added, and, inasmuch as new claims 91-102 include all the limitations of composition claim 57, they are eligible for rejoinder and examination upon allowability of claim 57.

Thus, claims 57, 63, 64, 66, 68-71 and 87-102, as shown above, are pending.

Specification

The disclosure was objected to for containing an embedded hyperlink. (Office Action, paragraph 2). Applicants note that the specification was amended, in a Response mailed on October 26, 2004, to remove the embedded hyperlink. Therefore, the objection can be withdrawn.

Sequence Listing

The specification was also objected to for failing to comply with the sequence disclosure requirements of 37 C.F.R. § 1.821(a)(1) and (a)(2). (Office Action, paragraph 3).

Applicants have amended the specification herein to provide a sequence identifier (SEQ ID NO:39) for the nuclear localization sequence shown on page 28, lines 6-7. A revised sequence listing including SEQ ID NO:39 is attached hereto.

The content of the copy in computer readable form is identical to the content of the paper copy of the sequence listing as submitted herewith and no new matter has been introduced.

Claim Objections

Claim 68 was objected to under 37 C.F.R. § 1.75(c) as improperly depending from a canceled claim. (Office Action, paragraph 4).

Applicants thank the Examiner for the careful attention paid to the claims and have amended claim 68 to depend from pending claim 66, thereby obviating the objection.

Rejections Withdrawn

The rejection of claims 57, 62-68, 70 and 71 under 35 U.S.C. § 102(e) as allegedly anticipated by Cox III *et al.* has been withdrawn. (Office Action, paragraph 5). The rejection of claim 60 under 35 U.S.C. § 102(e) as allegedly anticipated by Cox III *et al.* in view of Neely *et al.* has also been withdrawn in view of the cancellation of claim 60. (Office Action, paragraph 6).

35 U.S.C. § 102(b)

Claims 57, 63, 64, 66, 68, 70, 88 and 89 were rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Crossley *et al.* in light of Chen *et al.* and Morceau. (Office Action, paragraph 8). The Office Action states that Crossley teaches that (1) the

promoter of the EKLF gene contains a GATA-1 binding site, (2) GATA-1 activates the EKLF promoter, and (3) expression of GATA-1 from a polynucleotide introduced into NIH3T3 cells (in which the EKLF promoter is inactive) activates the EKLF promoter in those cells. *Id.* From these facts, the Office appears to conclude that Crossley teaches binding of GATA-1 to a site in the EKLF promoter. The Office then cites Chen to support the assertion that the EKLF promoter inherently comprises two DNase I hypersensitive sites, the distal-most of which is bound by GATA-1. *Id.*

Applicants traverse the rejection. Crossley, alone or in light of Chen and Morceau, does not anticipate the claimed complexes or cells comprising these complexes.

The pending claims relate to complexes between an exogenous molecule and a binding site in cellular chromatin, wherein the binding site lies in a region that is sensitive to a probe of chromatin structure. As stated in the Office Action, Crossley teaches nothing more than the transactivation of the EKLF promoter by GATA-1. Although it is possible that such transactivation is mediated by binding of GATA-1 to the EKLF promoter, such is not the only possible mechanism. For example, GATA-1 could activate the EKLF promoter indirectly, by binding to and activating a third gene, whose product activates the EKLF promoter.

However, if it is assumed, for the purposes of argument, that Crossley does, in fact, teach binding of GATA-1 to the EKLF promoter in NIH3T3 cells, such disclosure still fails to anticipate the claimed subject matter, since Crossley is silent with respect to whether any hypothetical GATA-1 binding site in the EKLF promoter is sensitive to a probe of chromatin structure. Chen's disclosure of two erythroid-specific DNase hypersensitive sites in a chromosomal EKLF gene in the murine 32DEpo1 cell line does nothing to show that any GATA-1 binding sites that may have been disclosed by Crossley are inherently nuclease sensitive, for at least two reasons.

First, Chen assayed DNase hypersensitivity of endogenous chromosomal EKLF sequences, while Crossley's transactivation experiments were conducted using an extrachromosomal plasmid containing an EKLF promoter. As was known in the art at the priority date, extrachromosomal DNA constructs such as those used by Crossley have a different chromatin structure than do endogenous chromosomal sequences. *See, for*

example, Smith & Hager (1997) *J. Biol. Chem.* **272**:27493-27496 (reference C3 of IDS attached hereto). Thus, Chen's disclosure of DNase hypersensitive sites in a chromosomal EKLF promoter provides no information about the chromatin structure of the extrachromosomal EKLF promoter sequences assayed by Crossley.

A second reason that Chen's findings are irrelevant to the chromatin structure of the EKLF sequences studied by Crossley is that Chen and Crossley studied different regions of the EKLF promoter. Crossley studied a region extending from -353 to +34, with respect to the transcription start site. (See Figures 1 and 2 of Crossley) Within this proximal promoter region, three GATA-1 binding sites, at approximately -45 (site 1), -60 (site 2) and -110 (site 3), were identified. By contrast, Chen studied more distal regions of the EKLF promoter. See, e.g., Chen at page 25031, second column, final paragraph:

Previous analyses had focused on the proximal EKLF promoter and revealed that the GATA site at -60 and the CCAAT element at -45 are important for activity in erythroid cells [citation to Crossley]. . . . However, the present studies use chromatin accessibility as a more global approach to find distal cis-elements that play a role in EKLF transcriptional activation. . . . the present studies have directed us to a distal 49-bp region within an erythroid-specific hypersensitivity domain that, in conjunction with the tissue-specific proximal EKLF promoter, plays a critical role in generating high levels of expression.

See also Chen at page 25032, second column, final paragraph:

Previously it had been shown that GATA and CP1 sites, located within 90 base pairs of the EKLF transcription initiation site, are important for activity in transient assays [citation to Crossley]. We wished to ascertain whether more distal elements could augment the level of transcriptional activity seen with this minimal promoter.

In fact, the two erythroid-specific DNase hypersensitive sites in the chromosomal EKLF gene identified by Chen were located at -700 and -300 (Chen at page 25033, paragraph bridging first and second columns), well outside of the region containing the three GATA-1 binding sequences identified by Crossley, the most distal of which was

located at approximately -110 (see above).¹ Moreover, the 49-bp region discussed above lies within the more distal of these two DNase hypersensitive sites, located at approximately -700. See Chen at page 25034, second column, first full paragraph, final sentence: "These analyses suggest that full EHS1 activity is localized to the 49-bp sequence between -715 and -666 of the EKLF promoter." Thus, inasmuch as Chen analyzed different sequences, his disclosure of hypersensitive sites within those distal sequences is uninformative with respect to the chromatin structure of the proximal sequences studied by Crossley.

Morceau, in stating that GATA-1 contains cysteine-rich motifs that are reminiscent of a zinc finger structure (Morceau at page 542, first full paragraph), adds nothing to further characterize the chromatin structure of any hypothetical GATA-1 binding site disclosed by Crossley.

Thus, the cited art does not show the binding of an exogenous molecule to a site in cellular chromatin that is sensitive to a probe of chromatin structure. Accordingly, the rejection should be withdrawn.

Finally, Chen's DNaseI hypersensitive site analyses does not support any type of inherent anticipation rejection, which might be based on the assumption that an exogenous polypeptide will necessarily and inevitably form a complex only with chromosomal cellular chromatin that is accessible to DNaseI.

In this regard, Applicants have previously shown that exogenous polypeptides do not necessarily and inevitably bind to DNase I hypersensitive sites. *See*, Response filed December 22, 2005, including the discussion of Zhang *et al.* (reference C5 of the IDS attached hereto), in which it was established that binding of an exogenous zinc finger polypeptide (and subsequent regulation of gene expression by the zinc finger polypeptide) does not occur solely at DNase I hypersensitive sites. In addition, Applicants have also previously shown that proteins are capable of binding to non-accessible chromatin. *See*, Response filed July 6, 2005, including the discussion of Wong *et al.* and Cirillo *et al.* (references C4 and C1 of IDS attached hereto). Thus, the

¹ Two additional, non-erythroid-specific, hypersensitive sites identified by Chen were located at -8 kb and +5.5 kb, even farther from the sequences characterized by Crossley

evidence of record clearly establishes that polypeptides (be they exogenous or endogenous) are not limited to binding only to accessible sequences. Accordingly, neither Chen's DNase I hypersensitive site mapping, nor any other analysis of the chromatin structure of the-EKLF gene, can support a rejection based on alleged inherent anticipation of the pending claims by Crossley.

For all of the foregoing reasons, withdrawal of the rejection is respectfully requested.

35 U.S.C. § 103

Crossley in view of Chen

Claims 57, 66 and 71 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Crossley in view of Chen. (Office Action, paragraph 11). In support of the rejection, the Office states, in part, that it "would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the complex of Crossley et al. by substitution of the human EKLF gene of Chen et al. because Chen et al. shows the two genes are highly conserved and the substitution would allow for further research on transcriptional control of the human EKLF gene." *Id.*

Applicants respectfully traverse the rejection. For the reasons noted above, if one assumes that Crossley describes a complex between an exogenous polypeptide and a binding site,² Chen fails to show that such a complex is present in a region of cellular chromatin that is sensitive to a probe of chromatin structure, as claimed.

Applicants also traverse the assertion that the motivation to combine Crossley and Chen is to "allow for further research on transcriptional control of the human EKLF gene." It is axiomatic that the motivation to combine references cannot derive from a high level of skill in the art. *See, also, In re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002) and *In re Rouffet* 47 USPQ2d 1453 (Fed. Cir. 1998). Here, the motivation to combine is essentially that there is a "high level of skill in the art. Furthermore, both Crossley and Chen used the murine gene as a model system to study EKLF transcriptional control.

² Although Crossley's results may suggest such complex formation, he fails to provide explicit disclosure of the formation of a complex

Nothing in Chen's comparison of the human and murine upstream sequences (Figure 2 of Chen) suggested that the human EKLF gene be substituted for the murine gene in their experiments on EKLF transcriptional control. Indeed, the comparison with the human gene was used by Chen to validate his results obtained with the mouse gene, prior to conducting further experiments on the mouse gene. (See Chen at paragraph bridging pages 25033-25034). Therefore, the motivation alleged by the Office to combine Crossley and Chen, namely further research on transcriptional control, is not present in the references or in the art as a whole.

In addition, Applicants reiterate that the motivation to combine cannot derive from the assumption that a polypeptide will bind cellular chromatin only at DNaseI hypersensitive sites. As the evidence of record establishes, proteins do not necessarily bind to chromatin exclusively in DNaseI hypersensitive sites. *See, above* and references C1, C3, C4 and C5 of attached IDS. Thus, the motivation to combine references cannot derive from the erroneous assumption that a polypeptide will necessarily bind to chromatin only at a DNaseI hypersensitive site. *See, also, In re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002) and *In re Rouffet* 47 USPQ2d 1453 (Fed. Cir. 1998).

In sum, the skilled artisan would have not have been motivated by any combination of Crossley and Chen to form a complex comprising an exogenous polypeptide and a site in cellular chromatin as claimed and, accordingly, the obviousness rejections cannot be sustained.

Finally, Applicants note that claim 71 recites a human cell. Both Crossley and Chen used murine cells. Crossley used MEL and NIH3T3 cells. See Crossley at page 15440, second column, fourth full paragraph and Figures 2 and 6. MEL is a murine erythroleukemia cell line and NIH 3T3 is a murine cell line, as evidenced by the attached page from the ATCC Web site, reference C6 of the IDS attached hereto. Chen used 32DEp01, CV1 and NIH3T3 cells. See Chen at page 25032, first column, first full paragraph. 32DEp01 is a murine cell line; see Chen at paragraph bridging pages 25032-25033. CV1 is an African green monkey line, as evidenced by the attached page from the ATCC Web site, reference C7 of the IDS attached hereto. As shown above, NIH3T3 is also a murine line.

Thus, there is no disclosure of human cells in either Crossley or Chen, and Chen's disclosure of a comparison between human and murine EKLF gene sequences does not provide disclosure of a human cell comprising a human EKLF gene. For this reason as well, the rejection should be withdrawn.

Crossley in view of Chen and in further view of Hays, Gregory or Greisman

Claims 57, 66 and 87 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Crossley in view of Chen as applied to claims 57, 66 and 71 above, and further in view of Hays. (Office Action, paragraph 12). Claims 57, 66 and 90 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Crossley in view of Chen as applied to claims 57, 66 and 71 above and further in view of Gregory. (Office Action, paragraph 13). In addition, claims 57, 66 and 69 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Crossley in view of Chen as applied to claims 57, 66 and 71 above and further in view of Greisman. (Office Action, paragraph 14). Crossley and Chen were cited as above. Hays was cited for reviewing the use of chemical probes to analyze chromatin structure. Gregory was cited for reviewing the use of restriction endonucleases as probes to analyze chromatin structure and Greisman was cited for teaching a strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites and for stating that zinc finger proteins provide means for developing plants with altered phenotypes.

For the reasons set forth above, Crossley and Chen do not describe, demonstrate or suggest the claimed subject matter. Therefore, there is no combination of Crossley, Chen and Hays; Crossley, Chen and Gregory; or Crossley, Chen and Greisman that renders the claimed subject matter obvious. Furthermore, the Office has provided no motivation to combine these references as suggested. Accordingly, the rejections should be withdrawn.

CONCLUSION

For the reasons set forth herein, Applicants believe that all pending claims are novel and non-obvious and are therefore in condition for allowance. Since all non-elected claims have been canceled, Applicants look forward to notification of allowance.

Respectfully submitted,

Date: August 22, 2006

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